Original article:

The effect of TAPI-1 treatment in non-small cell lung cancer cells

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Abstract

Lung cancer is one of the deadliest cancer types worldwide and is divided into 2 main types, small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). Non-small cell lung cancer is very common types of malignancy with low overall survival rate around 10%.The main problem in the treatment of NSCLC is still resistance to chemotherapy and metastasis. An increasing number of studies have indicated that many molecular mechanisms are dysregulated in NSCLC. Therefore, we used TAPI-1 (TNF-alpha protease inhibitor I) which is ADAM17/TACE inhibitor, and blocks shedding of cytokine receptors. ADAM17, also known as tumor necrosis factor-α (TNF-α) converting enzyme (TACE) is involved in multiple cell signaling pathways including Notch and EGFR. Moreover, it has been suggested that dysregulation of ADAM17 may be intricate in tumorigenesis and play a role in tumor development. Thus, the present study aimed to investigate whether TAPI-1 treatment of A549 NSCLC cells could influence mRNA expression of Notch1 and EGFR, and whether it affects cells viability. Materials and Methods: We used A549 NSCLC cells to examine TAPI-1 ADAM17/TACE inhibition effect. Moreover, we aimed to investigate the mRNA level of EGFR and Notch1 before and after TAPI-1 treatment. Results: TAPI-1 reduced proliferation of A549 cells and affect EGFR and Notch1 expression. Conclusion: We recognized molecular mechanism in which both Notch1 and EGFR are most likely regulated by ADAM17 and treatment with TNF-alpha protease inhibitor I have impact on the viability of A549 NSCLC cells.

Keywords: TAPI-1, Notch, EGFR, NSCLC

Introduction

Non–small cell lung cancer (NSCLC) is the principal cause of cancer-related deaths in the world with low overall survival rate(1). Moreover, achieving effective therapy of NSCLC is delayed by frequent metastasis and recurrence(2). Therefore, the analysis of molecular mechanisms involved in tumorigenesis is crucial in the development of new therapeutic targets. ADAM17 is enzyme belongs to the ADAM protein family of disintegrins and metalloproteases and is expressed in the brain, heart, lung, kidney, and skeletal muscle and its manifestation vary during embryonic development and adult life(3). In the physiological condition it is associated with cell adhesion, cell-cell signaling and cell migration(4). It is also involved in numerous proteolytical processes that are important in tumor development, including lung cancer(5). Moreover, it is known that ADAM17 can trigger the EGFR and is involved in numerous downstream signaling pathways, including Notch(6). Therefore, the objective of the present study was to identify the effect of TAPI-1 inhibition in A549 cells. Consequently, we tested whether the inhibition with the above chemical implicates the viability of A549 cells. Furthermore, we checked the mRNA expression levels of the EGFR and Notch1, which belong to key transduction pathways and their dysregulated expression levels are hallmarks of non-small cell lung carcinoma. Collectively, these studies indicate that TAPI-1 influence A549 cells viability, as well as EGFR and Notch1 mRNA expression.

Materials and Methods

The A549 cell lines were used in the study. The cells belong to adenocarcinoma human alveolar basal epithelial cells. The A549 cells were got from the American Type Culture Collection (ATCC). A549 were cultured in RPMI-1640

containing 10% fetal bovine serum (FBS) and antibiotics. The cells were incubated at 37˚C in a humidified environment with 5% CO2. The cell culture reagents were purchased from ATCC.

RNA extraction and quality control

Total RNA was isolated from A549 cells using the mirVana miRNA isolation kit (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's protocol. The 100-µl resulting RNA extracts were stored at −80˚C prior to further processing. Quantity and quality of RNA was measured using aUV/VIS spectrophotometer NanoDrop 2000c (Thermo Fisher Scientific, Waltham, MA, USA). The level of integrity required for quantitation (RNA integrity number >7) was determined for the extracted total RNA using the Agilent RNA 6000 Nano Kit on a Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA, USA).

A total of 500 ng RNA was reverse transcribed into cDNA in a reaction with High Capacity RNA-to-cDNA Master Mix (Applied Biosystems; Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's protocol.

Quantitative polymerase chain reaction (qPCR)

The mRNA expression levels of Notch1 and EGFR were calculated in the A549 cell line using comparative qPCR. The TaqMan probes for both genes Notch1 and EGFR, respectively (Hs01062014_m1, Hs01076078_m1) and the TaqMan Assay Kit (all from Applied Biosystems; Thermo Fisher Scientific, Waltham, MA, USA) were used to perform PCR. The expression of the above-mentioned genes [by the change-in-cycling threshold ΔCq method](7) was calculated and normalized to ribosomal *18SRNA* gene expression (Hs99999901_s1 *18SRNA*). The following cycling conditions were used: 50˚C for 2 min; 95˚C for 10 min; 40 cycles of 95˚C for 15 sec and 60˚C for 60 sec. Each sample was analyzed in triplicate. All reactions were performed using the ABI PRISM® 7900HT Sequence Detection system (Thermo Fisher Scientific, Waltham, MA, USA).

Results

The mRNA level of EGFR was significantly downregulated, when related to untreated cells after treatment with 20 µM TAPI-1 inhibitor for 24h and 48h. The similar drop down of mRNA EGFR was visible after 40µM of treatment, although after 48h with the same concentration, mRNA EGFR elevated but not significantly (Figure1). Furthermore, we did the same experiment to evaluate the mRNA level of Notch1 (Figure2). We noticed that after 24h of drug application, mRNA of Notch1 slightly increased. Nevertheless, after 48 h, Notch1 expression was reduced. Although, only 40µM TAPI prompted to significant reduction of mRNA Notch1 (Figure2).We also evaluated the morphological changes in A549 cells, and it turned out, that the cells grew slower than before treatment and most of them detached the plate and apoptotic features were noticeable (Figure3).

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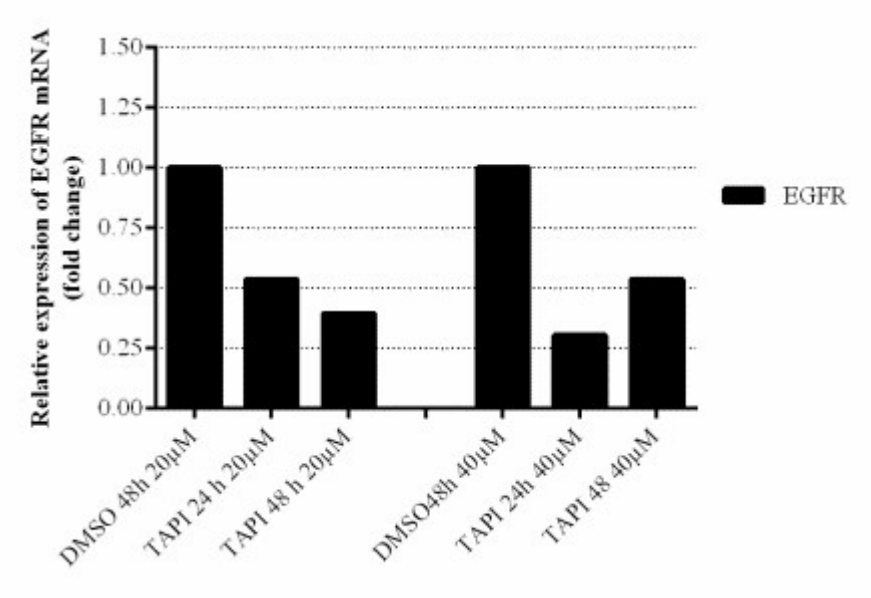


Figure 1. Relative expression of mRNA EGFR before and after TAPI treatment

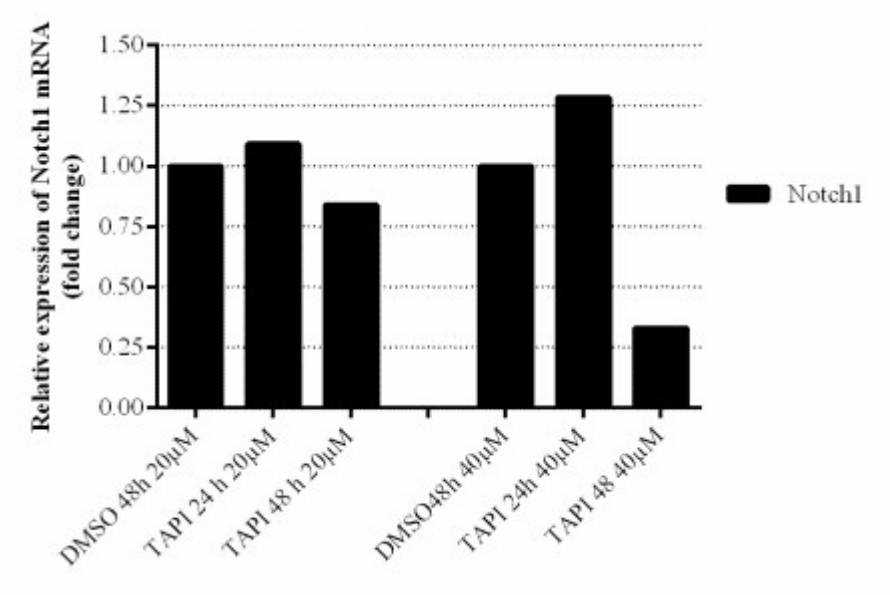
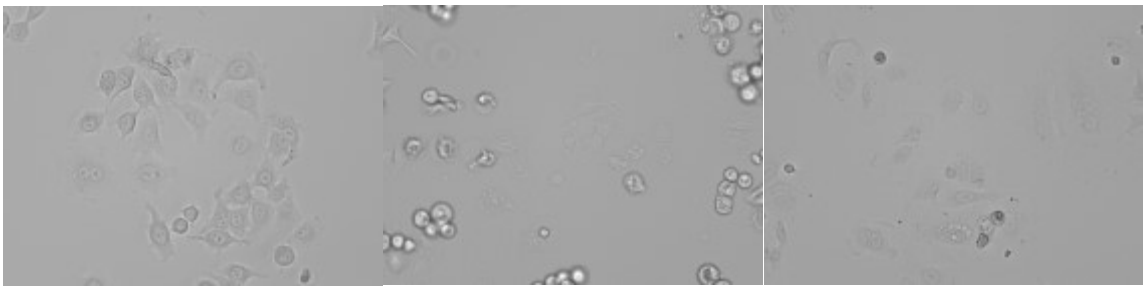


Figure 2. Relative expression of mRNA Notch1 before and after TAPI treatment



DMSO 24h, 20 μM TAPI-1 24h, 40 μM TAPI-1

Figure 3. The morphological changes in A549 cells before and after 20 μM and 40 μM TAPI-1 treatment for 24h. (20x magnification)

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Discussion

In the present study we aimed to examine the effect of TAPI-1 inhibition on A549 NSCLC cells. Therefore, we used TNF-alpha protease inhibitor I, which supposed to affect ADAM17 enzyme. The above-mentioned enzyme is important for cell survival and apoptosis and is very often deregulated in various types of tumor, including lung cancer (8). Moreover, it is described in the literature that ADAM17 affects EGFR and Notch signaling, which in turn are the key transduction pathways dysfunctional in NSCLC (9). Hence, we treated A549 cells with TAPI-1 for 24 and 48 h, after that we evaluate the viability of cells. It came out; that A549 cells grew slower than control, and some cancer cells indicated apoptotic features. Moreover, inhibition with TAPI-1 changed mRNA level of chosen genes. It caused a significant decrease of EGFR and a slight decrease of Notch1. Nevertheless, after 48h of treatment with 40μM TAPI-1, mRNA Notch1 also drops down. According to literature, there are some possible mechanisms for Notch-EGFR cooperation, either antagonistic or synergistic (10). Conferring some research studies, the expression of Notch-1 was upregulated in EGFR tyrosine kinase inhibitors (EGFR-TKISs) resistant lung cancer cells. Further, Notch-1 contributed to the achievement of the epithelial–mesenchymal transition (EMT) phenotype, which was associated with developed resistance to EGFR-TKISs (11). Another study showed that whereas inhibition of EGFR leads to reduction in tumor cell number, it also leads to a strong activation of the Notch pathway (12). Moreover, following the results of Baumgart A et al., Notch1 silencing caused a reduction of EGFR expression in all analyzed NSCLC cell lines. Besides, connection between Notch and EGFR, the researchers indicated that targeting Notch1 or ADAM17 led to cell death, whereas EGFR reduction caused cell cycle arrest. Further analysis conducted on primary human tissue by above mentioned researchers, revealed a significant correlation between ADAM17, Notch1 signaling, and high EGFR expression levels (6). Other investigators also tend to find a connection between TAPI-1 inhibition and Notch. According to Murthy et al., TAPI-1 treatment similarly led to the reduction of the active form of Notch as measured by immunoblotting (13). Moreover, previous studies indicate that ADAM17 is necessary for ligand-independent Notch activation (14,15).

Taking together, based on our results and available data, it seems that there is a connection between Notch and EGFR in NSCLC cells. Although further investigation is needed, ADAM17 directed therapy could be a good direction to evaluate possible mechanisms of Notch-EGFR crosstalk in NSCLC. Therefore, research on mechanisms that evaluate both signaling pathways in NSCLC seems to be justified in practice, since the results of experiments leading to simultaneous inhibition of both paths are already very promising (10).

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